Differential Effects of Two Lots of Aroclor 1254: Congener-Specific Analysis and Neurochemical End Points

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Aroclor 1254 is a widely studied commercial polychlorinated biphenyl (PCB) mixture which, by definition, contains 54% chlorine by weight. Recent reports indicate substantial differences in the congener composition among Aroclor lots and hence their biologic effects. We designed the current study to compare the effects of two lots of Aroclor 1254 (lots 6024 and 124-191). We analyzed these two lots for PCB congeners, polychlorinated dibenzofurans (PCDFs), polychlorinated naphthalenes (PCNs), and polychlorinated dibenzodioxins (PCDDs). We used previously established techniques for analyzing intracellular Ca^{2+} buffering and protein kinase C (PKC) translocation to test their biologic activity in neuronal preparations. PCB congener-specific analysis indicated that *ortho* and non-*ortho* congeners in these two lots varied in their percent contribution. Among all congeners, the percentages of non-*ortho* congeners (PCBs 77, 81, 126, and 169) were higher in lot 6024 (2.9% of total) than in lot 124-191 (0.02% of total). We detected no dioxins in these two lots (< 2 ppb). Although there are some differences in the congener composition, total PCNs were similar in both lots: 171 ppm in lot 6024 and 155 ppm in lot 124-191. However, total PCDFs were higher in lot 6024 (38.7 ppm) than in lot 124-191 (11.3 ppm). When we tested these two Aroclors on Ca^{2+} buffering and PKC translocation in brain preparations, the effects were significantly different. Although lot 124-191 was more potent on PKC translocation than lot 6024, lot 6024 was slightly more active on Ca^{2+} buffering than lot 124-191. These effects could not be attributed to the differences in the percentage of non-*ortho* congeners or PCDFs because they were inactive on these two parameters. The effects could not be attributed to PCNs because the levels were almost similar. The effects seen with two lots of Aroclor 1254 in neuronal cells were also not predicted based on the TCDD toxic equivalents (TEQs), although TEQs predicted the effects on ethoxyresorufin-*O*-deethylase (EROD) or methoxyresorufin-*O*-deethylase (MROD) activities. It is possible that the differential effects seen in neuronal cells could be caused by differences in the composition of *ortho*-congeners in these two mixtures, because PCBs with *ortho*-lateral substitutions can exhibit different activities on the selected neurochemical end points. Because of these differential effects with different lot numbers, the composition of Aroclor mixtures used in investigations should be disclosed. *Key words:* Aroclor 1254, dioxins, polychlorinated biphenyls, protein kinase C, toxic equivalents. *Environ Health Perspect* 109:1153–1161 (2001). [Online 5 November 2001]
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Polychlorinated biphenyls (PCBs) belong to a large group of halogenated aromatic hydrocarbons and consist of 209 theoretically possible congeners with different numbers and positions of chlorines (*1,2*). These compounds were commercially produced as Aroclor mixtures in the United States by the chlorination of biphenyl, which produces technical mixtures containing a given chlorine content depending on the duration of the chlorination process. Although all 209 congeners can be synthesized, the reaction conditions in the commercial processes favor certain substitution reactions leading to particular composition of the technical mixtures, which are identified by the weight percentage of chlorine content. For example, Aroclor 1254 contains 54% chlorine by weight, as indicated by the last two digits in the numeric designation (*1–5*).

These compounds were used widely in industry as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and capacitors, flame retardants, plasticizers, and sealants and in carbonless copy paper because of their chemical and thermal stability, dielectric properties, and miscibility with organic compounds. These same properties have now contributed to their ability to cause environmental and human health problems (*6–9*). PCBs are distributed throughout the entire ecosystem including soil, air, and water (*10*). They are also highly likely to bioaccumulate in the food chain because of their lipophilicity, and they therefore belong to a class of environmental chemicals called persistent bioaccumulative toxicants (PBTs) (*11*).

PCBs have a wide range of effects in humans, including chloracne, diverse hepatic effects, decreased birth weight in the offspring of occupationally exposed mothers, decreased pulmonary function, eye irritation, subtle endocrine disturbances, cancer, and learning and memory deficits (*10–13*). Several of these effects have also been demonstrated in animals during adult and developmental exposure to commercial PCB mixtures such as Aroclor 1254 (*6,7,9,11, 14,15*). In animal models, some individual PCB congeners and commercial PCB mixtures have shown tumor-promoting activity (*16*). The information from these animal studies with commercial PCB mixtures has been used in the risk/exposure assessment of PCBs and related environmental chemicals (*2,15*). However, recent reports using high-resolution gas chromatography (HRGC) indicate substantial differences in the congener composition among Aroclor lots (*17,18*); hence their biologic effects could be different. Toxicity studies with Aroclor 1254 have produced varied results (*19,20*), but these differences were attributed to the animal species or dosing paradigm rather than to the composition of Aroclor 1254. We designed the current study to study the effects of two lots of Aroclor 1254 (lots 6024 and 124-191) obtained from AccuStandard

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(New Haven, CT, USA) on the neurochemical end points that were previously reported to be sensitive to PCBs in neuronal preparations (*21–23*) and to compare the effects with the PCB congener composition and other contaminants in these two lots of Aroclor 1254. These two lots of Aroclor 1254 have been used widely by investigators for the last several years.

Materials and Methods

Chemicals. Aroclor 1254 (> 99% purity) with lot numbers 6024 and 124-191 were purchased from AccuStandard. For neurochemical experiments, we prepared stock solutions of two Aroclor 1254 lots by dissolving them in dimethyl sulfoxide (DMSO). A 2-µL aliquot of stock solution (different concentrations) was added to the buffer to yield the desired final concentrations. DMSO (2 µL/mL) had no significant effect either on $^{45}Ca^{2+}$ uptake or ^{3}H-phorbol ester binding.

Animals. Timed pregnant female (13 days of gestation) and adult male (90–120 days) Long-Evans rats were obtained from Charles River Laboratory. Pregnant dams were housed individually and adult rats were housed two per cage in Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facilities. Food (Rodent Chow; Purina, St. Louis, MO) and water were provided *ad libitum.* Temperature was maintained at 21 ± 2°C and relative humidity at 50 ± 10% with a 12-hr light/dark cycle (0700–1900 hr).

PCB congener-specific analysis of two lots of Aroclor 1254 with two lot numbers. We performed congener-specific analysis of PCBs according to previously established method (*24–26*).

Non-*ortho*, coplanar PCBs must be isolated from other dominant *ortho*-PCBs for their trace level determination. This is achieved by the use of Cosmosil 5-PYE column-HPLC method, as described in Kannan et al. (*26*). HPLC was performed with Pump-Constametric III with Rheodyne injector (LDC Analytical Gmbh, GH Friedrich, Germany) and the flow rate was 1 mL of hexane per minute on Cosmosil 5-PYE column [2-(1-pyrenyl) ethyldimethylsilylated silica gel], 250 × 4.6 mm, particle size 5 mm (Nacalai Tesque, Kyoto, Japan). From the stock solution (~1 ng/µL), 100 µL was injected and the eluates were collected in 4 fractions of 16 mL. The dead volume was 3.5 mL. The first fraction: 3.5–5.0 mL; second fraction 5–7.5 mL; third fraction 7.5–16 mL. These fractions were subsequently cooled (0°C) and concentrated using vacuum flash evaporator (flushing with N_2) and analyzed in high-resolution multidimensional gas chromatography-electron capture detector (HRMDGC-ECD).

This was performed using Fison 8000 GC-ECD (Fison Instruments Gmbh, Goettingen, Germany) with moving capillary stream switching technique. The gas chromatograph was equipped with a column injector, two columns in two independent ovens and two Ni^{63} electron capture detectors. An apolar SE-54 (50 m length, 0.25 mm internal diameter) was placed in the first oven, and a more polar OV-210 (30 m length, 0.32 mm internal diameter) in the second one. Gas pressure (H_2) was 1.1 bar and 0.6 bar. Temperature programming conditions were as follows: first column from 50°C (1 min) to 160°C (5 min) up to 250°C at 5°C per min and the second column at 100°C until 20 min after injection; then temperature increased to 240°C at 5°C per min. The instrument was well tuned to inject the sample three times and the average was taken. Because non-*ortho* PCBs contribute to many of the toxic equivalents (TEQ) values, we extracted triplicate samples. The variability was always less than 10%. Standardization of this method was done using artificially created PCB mixture containing 46 individual congeners from non-*ortho* to tetra-*ortho* chlorine substituted PCBs (Table 1).

We determined coplanar PCBs exclusively from the III fraction of PYE column chromatography using individual PCBs 77, 81, 126, and 169 standards (99.9% pure). Any possible coelution from other congeners

Table 1. Composition of polychlorinated biphenyls in the standard solution, their structures, IUPAC numbers, number of *ortho*-chlorine substitution, and the coeluting congeners.

IUPAC no.	Structure	Concentration (pg/µL)	Fraction	No. of *ortho* Cl	Coelution (IUPAC nos.)
8	2,4′	17.5	I	1	5
18	2,2′,5	12.0	I	2	17,15
28	2,4,4′	9.1	I	1	31
31	2,4′,5	10.6	I	1	28
44	2,2′,3,5′	10.7	I	2	
49	2,2′,4,5′	12.1	I	2	
52	2,2′,5,5′	17.3	I	2	
66	2,3′,4,4′	11.0	I	1	95
70	2,3′,4′,5	13.9	II	1	
74	2,4,4′,5	9.7	II	1	
77	3,3′,4,4′	10.3	III	0	110
81	3,4,4′,5	10.6	III	0	
92	2,2′,3,5,5′	10.6	I	2	
95	2,2′,3′,5,6	11.4	II	3	66
99	2,2′,4,4′,5	9.7	I	2	
101	2,2′,4,5,5′	18.5	I	2	90
105	2,3,3′,4,4′	11.0	III	1	153,132
110	2,3,3′,4′,6	11.1	II	2	77
118	2,3′,4,4′,5	17.0	II/III	1	123,149
123	2′,3,4,4′,5	11.8	III	1	149,118
126	3,3′,4,4′,5	11.0	III	0	129,178
128	2,2′,3,3′,4,4′	14.2	II	2	
129	2,2′,3,3′,4,5	9.7	I	2	126,178
132	2,2′,3,3′,4,6′	11.1	II	3	105,153
137	2,2′,3,4,4′,5	12.0	II	2	176
138	2,2′,3,4,4′,5′	27.6	II	2	158,160
141	2,2′,3,4,5,5′	9.6	I	2	179
149	2,2′,3,4′,5′,6	12.1	I	3	123,118
153	2,2′,4,4′,5,5′	16.7	II	2	105,132
156	2,3,3′,4,4′,5	9.4	III	1	171,202
157	2,3,3′,4,4′,5′	4.3	III	1	173,201
169	3,3′,4,4′,5,5′	9.2	III	0	
170	2,2′,3,3′,4,4′,5	13.4	III	2	190
174	2,2′,3,3′,4,5,6′	9.4	II	3	
177	2,2′,3,3′,4′,5,6	9.5	II	3	
178	2,2′,3,3′,5,5′,6	9.8	II	3	129,126
179	2,2′,3,3′,4,6,6′	9.3	II	4	141
180	2,2′,3,4,4′,5,5′	32.6	II	2	
183	2,2′,3,4,4′,5′,6	10.3	I	3	
187	2,2′,3,4′,5,5′,6	16.1	I	3	
189	2,3,3′,4,4′,5,5′	3.4	III	1	
190	2,3,3′,4,4′,5,6	10.4	III	2	170
191	2,3,3′,4,4′,5′,6	2.6	III	2	
194	2,3,3′,4,4′,5,5′,6	12.6	III	2	
199	2,2′,3,3′,4,5,6,6′	9.3	II	4	
202	2,2′,3,3′,5,5′,6,6′	10.6	I	4	156,171

PYE column HPLC separation of these congeners is shown in the column "Fraction." Fraction I, 3.5–5.0 mL; fraction II, 5.0–7.5 mL; fraction III, 7.5–16 mL hexane.

was monitored using a HRMDGC-ECD. We determined the remaining PCB congeners applying our routine procedure to environmental samples. Using the specially prepared technical standard, we determined the presence of every PCB congener. When a congener was present at levels < 0.05%, we considered it undetectable. Heart-cuts were made for those congeners that coeluted usually (*27*).

Analysis of PCDFs, PCDDs, and PCNs in two lots of Aroclor 1254. We performed the polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and polychlorinated naphthalene (PCN) analyses as per the modified methods described previously (*28,29*). Isomer-specific analyses were done using HRGC (HP6890) low-resolution mass spectroscopy (Hewlett-Packard 5973MS; Hewlett Packard, Tokyo, Japan).

PCB samples (50–100 mg of Aroclor 1254 with lot numbers 6024 and 124-191) dissolved in 1–5 mL hexane were chromatographed on basic alumina (300 mesh, 5 g; Merck. Ltd., Darmstadt, Germany) activated overnight at 130°C. After the initial absorption, 150 mL of hexane was passed through the column to remove most of the PCBs, followed by 100 mL of dichloromethane:hexane (1:1) at 1 drop/sec flow rate. The second fraction was concentrated to 1 mL and applied to activated charcoal/silica gel minicolumn. The minicolumn was washed with 40 mL toluene in the reverse direction, and 30 mL 5% methylene chloride/hexane, followed by 10 mL hexane in the normal direction, before use. After the initial absorption, the column was washed with 10 mL of hexane and then 30 mL of 5% methylene chloride/hexane to remove nonplanar PCBs. The final eluate that was obtained using 40 mL of toluene in the reverse direction was microconcentrated to 0.1 mL and applied for determination by HRGC-LRMS. We used an HP6890 (gas chromatograph)–HP5973 (mass spectrometer) (Hewlett Packard) equipped with SP-2331 fused silica capillary column (60 µm internal diameter) to analyze tetra- to hexachlorinated PCDD/PCDFs, and a DB-5 column (30 µm internal diameter) to determine hepta- and octachlorinated isomers. The MS was equipped with a quadrapole system and with an electron impact ionization source.

The identification of PCDFs and PCDDs was based on the fact that signals were observed at characteristic mass numbers (*m/z*) at proper retention times and in proper ratio of the M+ and (M+2) cluster ions in mass fragmentography. We identified PCDF isomers with reference to authentic standards from Wellington Laboratories Ltd. (Ontario City, Ontario, Canada). We used EPA-1613PAR for the 2,3,7,8-substituted isomers, and we identified all the other isomers reported according to Ryan et al. (*30*). We used EPA-1613LCS and EPA-1613CSS as an internal standard and a syringe spiking standard. Recoveries of tetra-CDD, penta-CDD, hexa-CDD, hepta-CDD and octa-CDD congeners through the analytic procedures were 66–140%, 83–129%, 70–112%, 62–121%, and 41–95%, respectively. Recoveries of PCN congeners have been reported earlier (*31*). PCN congener 1,2,3,4-tetra-CN was present in blank at trace amounts and therefore was not quantified.

Neurochemical end points. Fractionation of adult rat cerebella to obtain microsomes and mitochondria was performed according to Cotman and Matthews (*32*). We determined intracellular Ca^{2+} buffering by measuring the uptake of $^{45}Ca^{2+}$ by microsomes and mitochondria as outlined by Moore et al. (*33*).

We isolated granule cells from cerebellum of 7-day-old rats by the enzymatic disruption of cells as outlined by Gallo et al. (*34*) with modifications (*35*). These cells were maintained for 7 days *in vitro* in culture and used for protein kinase C (PKC) translocation studies. We determined PKC translocation by measuring ^{3}H-phorbol ester binding according to Vaccarino et al. (*36*).

Statistics. We analyzed the neurochemical data by two-way analysis of variance (ANOVA) with lot number as one factor and the concentration as the other followed by Dunnett's post-hoc test. All analyses were performed with PROC GLM of SAS software (*37*). We calculated the IC_{50} (concentration that inhibits control activity by 50%) and E_{50} (concentration that increases control activity by 50%) values for $^{45}Ca^{2+}$ uptake and ^{3}H-phorbol ester binding, respectively, from the regression line fit to the linear portion of the curve using GraphPad Instat Software (GraphPad Instat Software Inc., San Diego, CA, USA). We compared the IC_{50} and E_{50} values between the two lots using Student's *t*-test. The accepted level of significance was set at $p < 0.05$.

Results

Composition of PCB congeners and contaminants such as PCDFs, PCDD, and PCNs in two lots of Aroclor 1254. A definitive statistical analysis of the quantitative performance of this congener-specific analysis requires detailed regression analysis of all standards, analysis of multiple replicates of sample, and multiple replicate injections. Time constraints, cumbersome heart-cut analysis of non-*ortho* PCB congeners, and tedious analyses of contaminants precluded such analyses. The quantitative information provided in this article permits only a comparison of relative congener distribution in the two lots of Aroclor 1254.

The congener-specific analysis of Aroclor 1254 indicated that the composition of PCBs was different in both lots (Table 2). Some PCB congeners were higher in lot 6024, whereas some other congeners were higher in lot 124-191. PCBs 40, 47–48, 70, 74, 77, 81, 92, 99, 105, 110, 123, 126, 138, 156, and 157 were higher in lot 6024. On the other hand, PCBs 44, 49, 52, 66, 85, 97, 132, 135, (137+176), 149, 174, and 187 were higher in lot 124-191.

When PCB congeners were grouped based on the number of chlorines (mono- to nona-; Table 5), both lots had similar percentages of PCB congeners except the heptachlorinated ones, where lot 124-191 had higher levels (5.9%) than did lot 6024 (3.5%). When the congeners were grouped based on the number of *ortho*-chlorine substitutions, non-*ortho* PCBs were several-fold (about 150-fold) higher in lot 6024 than in lot 124-191. Mono-*ortho* PCBs were also significantly higher in lot 6024 (38% of total) than in lot 124-191 (24% of total). On the other hand, tri-*ortho* PCBs were significantly higher in lot 124-191 (21% of total) than in lot 6024 (14% of total), while di- and tetra-*ortho* PCBs were almost similar in both lots (Table 6).

PCDF composition was different in both lots of Aroclor 1254 as well. Lot 6024 had higher levels of total PCDFs as well as 2,3,7,8-PCDFs than did lot 124-191 (Tables 3 and 5). Based on the amount, total as well as penta-CDFs to octa-CDFs were higher in lot 6024 than in lot 124-191. The percentage of tetra-CDFs was lower and penta-CDFs was higher in lot 6024 than in lot 124-191. Dioxins were not detected in either lot of Aroclor 1254 (Tables 3 and 5). Total PCNs were similar in both lots: 171 pg/µg in lot 6024 versus 155 pg/µg in lot 124-191. However, penta- and hexa-PCNs were slightly higher in lot 6024, while hepta- and octa-PCNs were slightly higher in lot 124-191 (Table 4).

We calculated the TEQ values for both lots of Aroclor 1254 using the toxic equivalent factor (TEF) values from the World Health Organization (*38*). We calculated the total TEQ value for lot 124-191 as 39.42 µg/g, and the TEQ value for lot 6024 was 400.63 µg/g. The TEQ value for lot 6024 was 11 times higher than that of lot 124-191 (*39*).

Neurochemical effects of two lots of Aroclor 1254. Both lots of Aroclor 1254 inhibited microsomal $^{45}Ca^{2+}$ uptake in a concentration-dependent manner (Figure 1). Microsomal $^{45}Ca^{2+}$ uptake in control tissue is 50.5 ± 2.1 (mean ± SE) pmol/mg protein/min. The ANOVA indicated a significant interaction: The PCB levels were

plotted either as concentration (microgram per milliliter; $F_{5,36}$ = 8.41; p < 0.0001) or as TEQ (picograms per milliliter; $F_{2,30}$ = 52.05; p < 0.0001), suggesting that the response of two lots of Aroclor 1254 on microsomal $^{45}Ca^{2+}$ uptake is different in each case. The calculated IC_{50} values for microsomal $^{45}Ca^{2+}$ uptake were significantly different between two lots of Aroclor 1254; the difference was much greater when the values were transformed to represent TEQ (nanograms TEQ per milliliter) compared to the original concentrations (micrograms Aroclor 1254 per milliliter).

Both lots of Aroclor 1254 also inhibited mitochondrial $^{45}Ca^{2+}$ uptake in a concentration-dependent manner (Figure 2). Mitochondrial $^{45}Ca^{2+}$ uptake in control tissue was 11.2 ± 0.4 (mean ± SE) pmol/mg protein/min. The ANOVA indicated a significant interaction. Either the PCB levels were plotted as concentration (micrograms per milliliter; $F_{5,36}$ = 4.04; p < 0.0052) or as TEQ (picograms per milliliter; $F_{2,30}$ = 46.3; p < 0.0001), suggesting that the response of two lots of Aroclor 1254 on mitochondrial $^{45}Ca^{2+}$ uptake is different in each case. The IC_{50} values for mitochondrial $^{45}Ca^{2+}$ uptake were not significantly different among the two lots of Aroclor 1254 when the levels were represented as concentration (micrograms per milliliter); however, they are significantly different when the values were transformed to represent TEQ (Table 7).

Glutamate (30 µM), which was used as a positive control, increased ^{3}H-PDBu binding by 2-fold, and this is in agreement with previous reports (*40,41*). Both lots of Aroclor 1254 significantly increased ^{3}H-PDBu binding in a concentration-dependent manner (Figure 3). ^{3}H-PDBu binding in control cultures is 479 ± 25 (mean ± SE) fmol/mg

Table 2. Congener-specific analysis of Aroclor 1254 with two different lot numbers (mg/g).

PCBs	IUPAC no.	No. of Cl	*o*-Cl	Lot 124-191	Lot 6024	PCBs	IUPAC no.	No. of Cl	*o*-Cl	Lot 124-191	Lot 6024
2	1	1	1	–	–	2,2′,6,6′	54	4	4	–	–
3	2	1	0	–	–	2,3,3′,4	55	4	1	–	–
4	3	1	0	–	–	2,3,3′,4′	56	4	1	–	–
2,2′	4	2	2	–	–	2,3,3′,5	57	4	1	–	–
2,3	5	2	1	–	–	2,3,3′,5′	58	4	1	–	–
2,3′	6	2	1	–	–	2,3,3′,6	59	4	2	–	–
2,4	7	2	1	–	–	2,3,4,4′	60	4	1	–	–
2,4′	8	2	1	–	–	2,3,4,5	61	4	1	–	–
2,5	9	2	1	–	–	2,3,4,6	62	4	2	–	–
2,6	10	2	2	–	–	2,3,4′,5	63	4	1	–	–
3,3′	11	2	0	–	–	2,3,5,6	65	4	2	–	–
3,4	12	2	0	–	–	2,3′,4,4′	{66 +	4	1	90.78	75.98
3,4′	13	2	0	–	–	2,2′,3,5′,6	95}	5	3		
3,5	14	2	0	–	–	2,3′,4,5	67	4	1	–	–
4,4′	15	2	0	–	–	2,3′,4,5′	68	4	1	–	–
2,2′,3	16	3	2	–	–	2,3′,4,6	69	4	2	–	–
2,2′,4	17	3	2	–	–	2,3′,4′,5	70	4	1	25.24	63.57
2,2′,5	18	3	2	–	–	2,3′,4′,6	71	4	2	–	–
2,2′,6	19	3	3	–	–	2,3′,5,5′	72	4	1	–	–
2,3,3′	20	3	1	–	–	2,3′,5′,6	73	4	2	–	–
2,3,4	21	3	1	–	–	2,4,4′,5	74	4	1	4.36	23.47
2,3,4′	22	3	1	–	–	2,4,4′,6	75	4	2	–	–
2,3,5	23	3	1	–	–	2′,3,4,5	76	4	1	–	–
2,3,6	24	3	2	–	–	3,3′,4,4′	77	4	0	0.01	27.20
2,3′,4	25	3	1	–	–	3,3′,4,5	78	4	0	–	–
2,3′,5	26	3	1	–	–	3,3′,4,5′	79	4	0	–	–
2,3′,6	27	3	2	–	–	3,3′,5,5′	80	4	0	–	–
2,4,4′	28	3	1	–	–	3,4,4′,5	81	4	0	0.01	0.28
2,4,5	29	3	1	–	–	2,2′,3,3′,4	{82 +	5	2	22.02	20.98
2,4,6	30	3	2	–	–	2,2′,3,5,5′,6	151}	6	3		
2,4′,5	31	3	1	–	–	2,2′,3,3′,5	83	5	2	4.29	4.30
2,4′,6	32	3	2	–	–	2,2′,3,3′,6	84	5	3	–	–
2′,3,4	33	3	1	–	–	2,2′,3,4,4′	85	5	2	7.16	–
2′,3,5	34	3	1	–	–	2,2′,3,4,5	86	5	2	–	–
3,3′,4	35	3	0	–	–	2,2′,3,4,5′	{87 +	5	2	42.21	47.22
3,3′,5	36	3	0	–	–	2,3,4,4′,6	115}	5	2		
3,4,4′	37	3	0	–	–	2,2′,3,4,6	88	5	3	–	–
3,4,5	38	3	0	–	–	2,2′,3,4,6′	89	5	3	–	–
3,4′,5	39	3	0	–	–	2,2′,3,4′,5	{90 +	5	2	72.28	61.60
2,2′,3,3′	40	4	2	2.11	3.36	2,2′,4,5,5′	101}	5	2		
2,2′,3,4	{41 +	4	2	9.71	9.81	2,2′,3,4′,6	91	5	3	20.24	22.54
2,3,4′,6	64}	4	2			2,2′,3,5,5′	92	5	2	28.34	36.00
2,2′,3,4′	42	4	2	–	–	2,2′,3,5,6	93	5	3	–	–
2,2′,3,5	43	4	2	–	–	2,2′,3,5,6′	94	5	3	–	–
2,2′,3,5′	44	4	2	25.18	10.46	2,2′,3,6,6′	96	5	4	–	–
2,2′,3,6	45	4	3	–	–	2,2′,3′,4,5	97	5	2	20.33	–
2,2′,3,6′	46	4	3	–	–	2,2′,3′,4,6	98	5	3	–	–
2,2′,4,4′	{47 +	4	2	–	5.96	2,2′,4,4′,5	99	5	2	24.97	37.10
2,2′,4,5	48}	4	2			2,3,3′,4,6	109	5	2	–	–
2,2′,4,5′	49	4	2	21.36	4.07	2,2′,4,4′,6	100	5	3	–	–
2,2′,4,6	50	4	3	–	–	2,2′,4,5,6′	102	5	3	–	–
2,2′,4,6′	51	4	3	–	–	2,2′,4,5′,6	103	5	3	–	–
2,2′,5,5′	52	4	2	35.06	7.73	2,2′,4,6,6′	104	5	4	–	–
2,2′,5,6′	53	4	3	–	–						

continued

protein/15 min. The ANOVA indicated a significant interaction: Either the PCB levels were plotted as concentration ($F_{4,49}$ = 5.94; p < 0.0006) or as TEQ ($F_{1,49}$ = 64.2; p < 0.0001), suggesting that the response of two lots of Aroclor 1254 on ^{3}H-PDBu binding is different in each case. The E_{50} values for ^{3}H-PDBu binding were significantly different between the two lots of Aroclor 1254; the difference was much greater when the values were transformed to represent TEQ than for the original concentrations (Table 7).

In general, the data from neurochemical end points indicate that the effects of two lots of Aroclor 1254 on intracellular Ca^{2+} buffering is comparable, but lot 124-191 was more effective on PKC translocation than was lot 6024. The potency difference between the two lots ranged from 1.2- to 2.5-fold when PCB levels were represented as concentration. However, this difference increased to several-fold (8- to 29-fold) when PCB levels were transformed to TEQs (Figures 1–3; Table 7).

Discussion

Health risks associated with exposure to PCBs and related chemicals have been assessed based on either total PCB concentrations or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) TEQs using the TEF concept when congener-specific data are available. U.S. EPA has adopted the TEF approach as an interim procedure that assumes additivity for the toxic effects of individual congeners in the mixtures and a common mechanism of toxic action (e.g., ability to interact with and activate the aryl hydrocarbon receptor). In addition, assessment of risks to humans is based on the reference doses (RfDs) derived from animal studies with commercial PCB mixtures (*15*).

Table 2 (*continued*).

PCBs	IUPAC no.	No. of Cl	No. of *o*-Cl	Lot 124-191	Lot 6024	PCBs	IUPAC no.	No. of Cl	*o*-Cl	Lot 124-191	Lot 6024
2,3,3′,4,4′	105	5	1	51.00	130.00	2,3,3′,4,4′,5′	157	6	1	0.36	26.30
2,3,3′,4,5	106	5	1	–	–	2,3,3′,4,4′,6	158	6	2	–	–
2,3,3′,4′,5	107	5	1	–	–	2,3,3′,4,5,5′	159	6	1	–	–
2,3,3′,4,5′	108	5	1	–	–	2,3,3′,4,5,6	160	6	2	–	–
2,3,3′,4′,6	110	5	2	76.57	87.15	2,3,3′,4,5′,6	161	6	2	–	–
2,3,3′,5,5′	111	5	1	–	–	2,3,3′,4′,5,5′	162	6	1	–	–
2,3,3′,5,6	112	5	2	–	–	2,3,3′,4′,5,6	163	6	2	–	–
2,3,3′,5′,6	113	5	2	–	–	2,3,3′,4′,5′,6	164	6	2	–	–
2,3,4,4′,5	{114 +	5	1	0.05	0.78	2,3,3′,5,5′,6	165	6	2	–	–
2′,3,3′,4,5	122 +	5	1			2,3,4,4′,5,6	166	6	2	–	–
2,2′,3,3′,4,6	131}	6	3			2,3′,4,4′,5,5′	167	6	1	–	–
2,3,4,5,6	116	5	2	–	–	2,3′,4,4′,5′,6	168	6	2	–	–
2,3,4′,5,6	117	5	2	–	–	3,3′,4,4′,5,5′	169	6	0	0.01	0.02
2,3′,4,4′,5	118	5	1	127.00	124.00	2,2′,3,3′,4,4′,5	170	7	2	4.02	3.39
2,3′,4,4′,6	119	5	2	–	–	2,2′,3,3′,4,4′,6	171	7	3	–	–
2,3′,4,5,5′	120	5	1	–	–	2,2′,3,3′,4,5,5′	172	7	2	–	–
2,3′,4,5′,6	121	5	2	–	–	2,2′,3,3′,4,5,6	173	7	3	0.77	1.74
2′,3,4,4′,5	123	5	1	0.57	2.14	2,2′,3,3′,4,5,6′	174	7	3	31.12	18.32
2′,3,4,5,5′	124	5	1	–	–	2,2′,3,3′,4,5′,6	175	7	3	–	–
2′,3,4,5,6′	125	5	2	–	–	2,2′,3,3′,4′,5,6	177	7	3	1.59	0.88
3,3′,4,4′,5	126	5	0	0.17	3.24	2,2′,3,3′,5,5′,6	178	7	3	–	–
3,3′,4,5,5′	127	5	0	–	–	2,2′,3,4,4′,5,5′	180	7	2	5.18	4.51
2,2′,3,3′,4,4′	128	6	2	7.04	9.67	2,2′,3,4,4′,5,6	181	7	3	–	–
2,2′,3,3′,4,5	129	6	2	8.70	9.34	2,2′,3,4,4′,5,6′	182	7	3	–	–
2,2′,3,3′,4,5′	130	6	2	–	–	2,2′,3,4,4′,5′,6	183	7	3	1.97	1.36
2,2′,3,3′,4,6′	132	6	3	33.03	25.82	2,2′,3,4,4′,6,6′	184	7	4	–	–
2,2′,3,3′,5,5′	133	6	2	–	–	2,2′,3,4,5,5′,6	185	7	3	–	–
2,2′,3,3′,5,6	134	6	3	–	–	2,2′,3,4,5,6,6′	186	7	4	–	–
2,2′,3,3′,5,6′	135	6	3	12.30	6.69	2,2′,3,4′,5,5′,6	187	7	3	3.51	0.39
2,2′,3,3′,6,6′	136	6	4	–	–	2,2′,3,4′,5,6,6′	188	7	4	–	–
2,2′,3,4,4′,5	{137 +	6	2	4.18	–	2,3,3′,4,4′,5,5′	189	7	1	–	–
2,2′,3,3′,4,6,6′	176}	7	4			2,3,3′,4,4′,5,6	190	7	2	–	–
2,2′,3,4,4′,5′	138	6	2	58.70	71.80	2,3,3′,4,4′,5′,6	191	7	2	–	–
2,2′,3,4,4′,6	139	6	3	–	–	2,3,3′,4,5,5′,6	192	7	2	–	–
2,2′,3,4,4′,6′	140	6	3	–	–	2,3,3′,4′,5,5′,6	193	7	2	–	–
2,2′,3,4,5,5′	{141 +	6	2	12.18	12.86	2,2′,3,3′,4,4′,5,5′	194	8	2	–	–
2,2′,3,3′,5,6,6′	179}	7	4			2,2′,3,3′,4,4′,5,6	195	8	3	–	–
2,2′,3,4,5,6	142	6	3	–	–	2,2′,3,3′,4,4′,5′,6	196	8	3	–	–
2,2′,3,4,5,6′	143	6	3	–	–	2,2′,3,3′,4,4′,6,6′	197	8	4	–	–
2,2′,3,4,5′,6	144	6	3	–	–	2,2′,3,3′,4,5,5′,6	198	8	3	–	–
2,2′,3,4,6,6′	145	6	4	–	–	2,2′,3,3′,4,5,6,6′	199	8	4	–	–
2,2′,3,4′,5,5′	146	6	2	10.29	8.44	2,2′,3,3′,4,5′,6,6′	200	8	4	–	–
2,2′,3,4′,5,6	147	6	3	–	–	2,2′,3,3′,4,5,5′,6′	201	8	3	–	–
2,2′,3,4′,5,6′	148	6	3	–	–	2,2′,3,3′,5,5′,6,6′	202	8	4	–	–
2,2′,3,4′,5′,6	149	6	3	41.76	14.37	2,2′,3,4,4′,5,5′,6	203	8	3	–	–
2,2′,3,4′,6,6′	150	6	4	–	–	2,2′,3,4,4′,5,6,6′	204	8	4	–	–
2,2′,3,5,6,6′	152	6	4	–	–	2,3,3′,4,4′,5,5′,6	205	8	2	–	–
2,2′,4,4′,5,5′	153	6	2	31.80	33.93	2,2′,3,3′,4,4′,5,5′,6	206	9	3	–	–
2,2′,4,4′,5,6′	154	6	3	–	–	2,2′,3,3′,4,4′,5,6,6′	207	9	4	–	–
2,2′,4,4′,6,6′	155	6	4	–	–	2,2′,3,3′,4,5,5′,6,6′	208	9	4	–	–
2,3,3′,4,4′,5	156	6	1	4.80	51.00	2,2′,3,3′,4,4′,5,5′,6,6′	209	10	4	–	–

No. of Cl, number of total chlorine substitutions; No. of *o*-Cl, number of *ortho*-chlorine substitutions. PCBs without any values are below the detection limit, < 0.05% (w/w). The PCB numbers are in agreement with IUPAC convention, but note the change in numbers for PCBs 199, 200, and 201 compared to Ballschmitter and Zell (*3*).

Recently, a new approach has been developed to assess the cancer risk from environmental PCB exposure, and this approach considers both toxicity and environmental processes to distinguish among environmental mixtures (*42*). Although current risk/exposure assessment is based on these approaches, several problems were identified with the recent literature. Several studies from our laboratory as well as others indicate that *ortho*-substituted PCBs that do not or weakly bind to aryl hydrocarbon receptors are highly active in neuronal and other preparations (*43–45*). Because *ortho*-PCBs are highly abundant in environmental samples (> 99%), lack of significant consideration of this group of PCBs in the TEF approach could lead to underestimation of risk associated with exposure to these PCBs. Likewise, RfDs derived from commercial mixtures might not be accurate because the pattern of relative proportions of PCBs in environmental mixtures is variable and does not resemble the composition of the original PCB mixtures that were released into the environment. Recent reports indicate substantial differences in the congener composition among Aroclor lots (*17,18*), so their biologic effects could be different (*19,20*). In the present study, we report a thorough PCB congener analysis along with the contaminants such as PCDFs, PCNs, and any dioxins in two different lots of Aroclor 1254 mixtures and their effects on selected neurochemical end points that were previously reported to be sensitive to PCBs in neuronal preparations (*21–23*).

Results from this study indicate that the composition of PCB congeners was significantly different between the two lot numbers of Aroclor 1254. We detected no dioxins in either lot (< 2 ppb), as anticipated. PCNs were similar in both lots. Other contaminants in this mixture, such as PCDFs, were detectable, mainly because PCDFs are produced often as co-contaminants in PCB preparation (*28*). In the present study, lot-to-lot differences in PCDF levels have been identified, with lot 6024 having 3.4 times more PCDFs than lot 124-191. Differences in the congener composition between different lots for a variety of Aroclor mixtures have been previously reported (*17,18*). However, this is the first detailed report of congener-specific analysis that includes contaminants in these two lots of Aroclor 1254, a widely used commercial mixture in the United States for conducting scientific research. Of the two lots of Aroclor 1254

Table 3. Concentration (ng/g; ppb) of PCDFs and PCDDs in Aroclor 1254 with two different lot numbers.

PCDFs/PCDDs	Lot 124–191	Lot 6024	PCDFs/PCDDs	Lot 124–191	Lot 6024T
Tetra-CDF (0.0 = ND; < 2.0 ppb)			Penta-CDF (0.0 = ND; < 2.0 ppb)		
1368	–	–	12348/12378	295.0	1920.2
1378/1379	10.0	–	12346	34.2	–
1347	–	–	12379	–	–
1468	–	–	12367	8.6	167.0
1247/1367	–	–	12469/12678	615.7	2838.6
1348	–	–	12679	–	–
1346/1248	109.9	7.6	12369	–	–
1246/1268	25.0	–	23468	81.2	250.5
1478/1369/1237	15.0	–	12349	–	–
1678/1234	–	–	12489	470.3	3757.0
2468/1238/1467/1236	54.9	–	23478	821.0	4049.2
1349	–	–	12389	–	–
1278	79.9	–	23467	162.5	751.4
1267/1279	30.0	30.4	Hexa-CDF (0.0 = ND; < 4.0 ppb)		
1469	–	–	123468	17.1	–
1249	84.9	68.5	134678	85.3	–
2368	–	–	134679	–	–
2467	15.0	15.2	124678	238.9	–
1239	–	–	124679	17.1	–
2347	89.9	289.2	123478/123479	1638.1	4571.4
1269	–	–	123678	733.7	3190.5
2378	129.9	350.1	124689	418.1	952.4
2348	719.2	730.6	123467	341.3	2142.9
2346	49.9	49.5	123679	–	–
2367	219.7	152.2	123469/123689	597.2	2238.1
3467	–	–	123789	–	–
1289	44.9	–	123489	443.7	2761.9
Penta-CDF (0.0 = ND; < 2.0 ppb)			234678	213.3	1333.3
13468	–	–	Hepta-CDF (0.0 = ND; < 4.0 ppb)		
12468	–	–	1234678	581.8	1506.5
13678	–	–	1234679	375.8	1286.8
13479	–	–	1234689	157.6	470.8
12368/13478	8.6	20.9	1234789	533.3	1459.4
12478	235.2	292.2	Octa-CDF (0.0 = ND; < 4.0 ppb)		
12479/13467	4.3	20.9	12346789	356.0	945.6
12467	94.1	41.7	PCDDs	ND	ND
14678/12347	102.6	41.7		< 2.0 ppb	< 2.0 ppb
13469	–	–			

ND, not detected.

Table 4. Concentration (ng/g; ppb) of PCNs in Aroclor 1254 with two different lot numbers.

PCNs	Lot 124–191	Lot 6024
3 Chlorines		
135	0.9	4.7
136	0.0	0.0
146/124	6.2	33.8
137	0.6	2.0
125	0.8	3.5
126	0.7	3.4
167/127	1.2	4.8
123	0.2	0.4
236	0.2	0.7
145/138	3.0	12.9
128	1.3	3.6
4 Chlorines		
1357	6.7	17.0
1246/1247/1257	56.9	120.2
1467	33.0	53.4
1367	1.0	2.3
1235/1256	12.1	21.9
1368/1358	16.8	55.8
1234/1236	1.3	2.7
1245/1237	12.8	14.4
1267	5.2	13.1
1248	19.2	43.1
1258	71.6	150.5
2367	0.0	0.0
1268	1.5	5.8
1458	36.2	46.3
1238	0.0	0.0
1278	44.1	15.2
5 Chlorines		
12357/12467	5709.1	2591.8
12457	423.0	105.8
12468	3005.4	735.0
12346	397.5	140.6
12356	761.8	227.5
12456	3183.9	1250.3
12367	59.7	8.4
12478	5586.3	1243.1
12358	2183.1	737.8
12368	0.0	0.0
12458	1550.3	880.3
12345	276.7	106.3
12378	122.9	89.6
6 Chlorines		
123467/123567	8899.8	6233.4
123457/123568	15260.7	10030.7
123578	31115.2	18089.3
124568/124578	24761.0	21068.5
123456	10914.4	6512.7
123458	3992.7	3736.1
123678	45.0	40.1
7 Chlorines		
1234567	18527.3	27277.7
1234568	26320.4	38534.3
8 Chlorines		
12345678	7799.1	14988.0

tested, lot 6024 has more non-*ortho* PCBs and PCDFs contributing to higher TEQ values. Recently, Frame (*18*) discussed the manufacturing process of several lots of Aroclor 1254 and the differences in the congener composition. Lot 124-191 of Aroclor 1254 has the typical PCB congener distribution. However, lot 6024 has unusually enhanced levels of non-*ortho* and mono-*ortho* congeners and PCDFs. Lot 6024 has been traced back to Monsanto lot KI-6024 and represents the late (1974–1976) production of Aroclor 1254s, which used a two-stage chlorination procedure (*18*). In the first stage, biphenyl was chlorinated to 42% chlorine content by weight as for Aroclor 1242 production and then fractionated to give a distillate that was sold as Aroclor 1016. In the second stage, the residue from the distillate was further chlorinated to 54% chlorine by weight, greatly increasing the levels of non-*ortho* and mono-*ortho* congeners with high TEF values (*18*). Caution should be used when comparing the results using this lot (lot 6024) with results from other lots of Aroclor 1254.

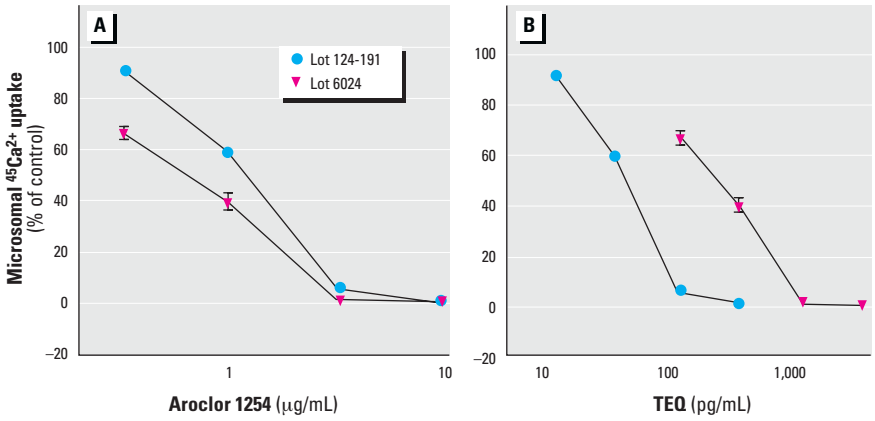

Figure 1. Inhibition of rat brain microsomal $^{45}Ca^{2+}$ uptake by Aroclor 1254 with two lot numbers. The $^{45}Ca^{2+}$ uptake was represented as percent of control (50.5 ± 2.1 pmol/mg protein/min). Values are mean ± SEM of four preparations, assayed in triplicate.

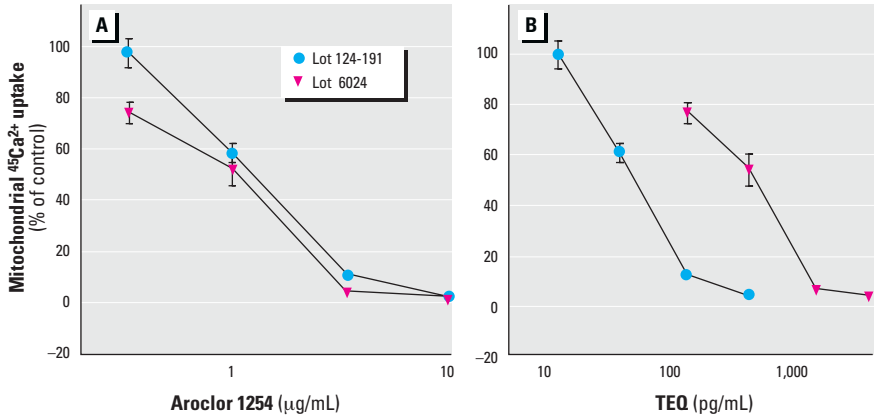

Figure 2. Inhibition of rat brain mitochondrial $^{45}Ca^{2+}$ uptake by Aroclor 1254 with two lot numbers. The $^{45}Ca^{2+}$ uptake was represented as percent of control (11.2 ± 0.4 pmol/mg protein/min). Values are mean ± SEM of four preparations, assayed in triplicate.

Table 5. Different PCB/PCDF/PCDD/PCN congeners based on the number of chlorines in the Aroclor 1254 mixtures with two different lot numbers.

Aroclor 1254 lot numbers	Units	Concentration					
		Mono, di, and tri	Tetra	Penta	Hexa	Hepta	Octa, nona
124-191							
PCBs	ng/μg	ND	168 (17.6)	504 (52.7)	228 (23.8)	56 (5.9)	ND
PCDFs	pg/μg	ND	1.68 (14.3)	2.93 (25.8)	4.74 (41.8)	1.65 (14.5)	0.36 (3.1)
2,3,7,8-PCDFs	pg/μg	ND	0.13 (2.6)	1.12 (22.6)	2.58 (52.3)	1.12 (22.5)	ND
PCDDs	pg/μg	ND	ND	ND	ND	ND	ND
PCNs	pg/μg	0.070	0.562	8.12	65.71	65.81	14.99
6024							
PCBs	ng/μg	ND	194 (18.6)	539 (51.6)	274 (26.3)	37 (3.5)	ND
PCDFs	pg/μg	ND	1.69 (4.4)	14.15 (36.6)	17.19 (44.4)	4.72 (12.2)	0.95 (2.4)
2,3,7,8-PCDFs	pg/μg	ND	0.35 (1.9)	5.97 (32.5)	9.09 (49.5)	2.97 (16.1)	ND
PCDDs	pg/μg	ND	ND	ND	ND	ND	ND
PCNs	pg/μg	0.015	0.319	23.26	94.99	44.85	7.80

ND, not detected. The numbers in parentheses indicate the percentage of total with respective groups (PCBs, PCDFs, 2378 PCDFs, or PCDDs).

The biologic activity of two lots of Aroclor 1254 was tested on two previously established neurochemical end points, intracellular Ca^{2+} buffering and PKC translocation. We selected these two end points on the basis of our previous work, where intracellular Ca^{2+} buffering by endoplasmic reticulum and mitochondria as well as PKC translocation were preferentially affected by *ortho*-substituted PCBs (*43*). Intracellular Ca^{2+} buffering is essential for maintaining normal calcium homeostasis (*46*). When intracellular free-Ca^{2+} levels increase, PKC may translocate from cytosol to the membrane, where it gets activated. Increases in intracellular free Ca^{2+} levels by PCBs have been reported by several investigators in several cell systems (*35,47,48*). In addition, increases in intracellular free-Ca^{2+} levels, inhibition of Ca^{2+} buffering, and PKC activation and translocation have been involved in the neurotoxicity of a variety of environmental chemicals (*49–51*). When these two lots of Aroclors were tested on Ca^{2+} buffering and PKC translocation in brain preparations, the effects were significantly different. Intracellular Ca^{2+} buffering by microsomes and mitochondria was significantly inhibited by both lots of Aroclor 1254 in a concentration-dependent manner. Lot 6024 seems to be more potent than lot 124-191. This difference in the potency increased several-fold when the concentration of Aroclor 1254 was transformed to TEQ values (derived from dioxin TEFs), demonstrating that the greater effect with lot 6024 is not caused by the greater aryl hydrocarbon-receptor binding activity alone. PKC translocation, measured

Table 6. PCB congeners (pg/ng) based on *ortho*-substitutions in the Aroclor 1254 mixtures with two different lot numbers.

PCBs	Total PCB congeners (pg/ng)	
	Lot 124-191	Lot 6024
Non-*ortho*	0.2 (0.02%)	30.74 (2.9%)
Mono-*ortho*	230.8 (24.1%)	393.4 (37.7%)
Di-*ortho*	514.5 (53.8%)	472.8 (45.3%)
Tri-*ortho*	202.7 (21.2%)	140.6 (13.5%)
Tetra-*ortho*	8.18 (0.85%)	6.43 (0.62%)

The numbers in parentheses indicate the percentage of total.

Table 7. Differential effects of two lots of Aroclor 1254 on intracellular Ca^{2+} buffering and PKC translocation in the brain.

Neurochemical end point	Concentration (μg/ml)		TEQ (ng/ml)	
	Lot 124–191	Lot 6024	Lot 124–191	Lot 6024
Intracellular Ca^{2+} buffering (IC_{50})				
Microsomes	1.65 ± 0.05	1.23 ± 0.05*	0.065 ± 0.002	0.493 ± 0.020*
Mitochondria	1.78 ± 0.07	1.47 ± 0.08	0.070 ± 0.003	0.589 ± 0.032*
PKC translocation (E_{50})	11.03 ± 1.29	28.01 ± 6.01*	0.435 ± 0.051	11.22 ± 2.41*

*Significantly different from lot 124-191 at $p < 0.05$. Values are mean ± SE of 4–6 dose–response experiments shown in Figures 1–3 (n = 4–6). IC_{50} values for Ca^{2+} buffering and E_{50} values for PKC translocation (^{3}H-phorbol ester binding) were calculated from the regression line fit to the linear portion of the data presented in Figures 1–3.

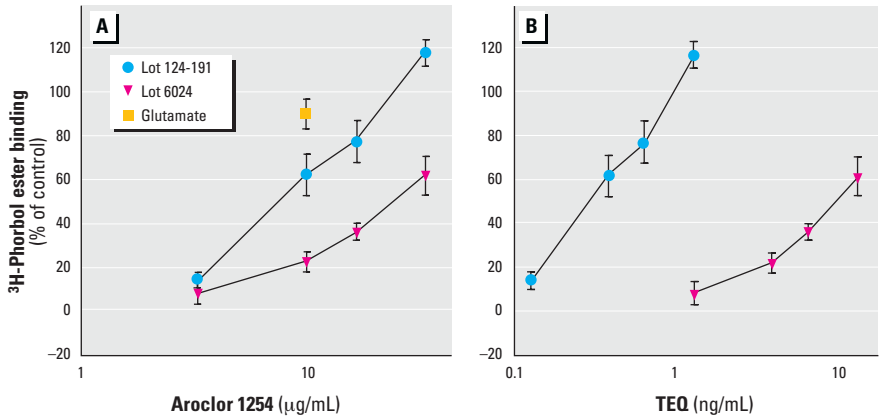

Figure 3. Increased ^{3}H-phorbol ester (PDBu) binding in rat cerebellar granule cells by Aroclor 1254 with two lot numbers. The ^{3}H-PDBu binding was represented as percent of control (479 ± 25 fmol/mg protein/15 min). Values are mean ± SEM of six preparations, assayed in triplicate.

as ^{3}H-phorbol ester binding, was significantly increased by both lots of Aroclor 1254; lot 124-191 was significantly more active than lot 6024. As seen with Ca^{2+} buffering, the difference in the potency also increased several-fold when the concentration of Aroclor 1254 was transformed to TEQ, suggesting that the dioxin-like congeners are not responsible for this effect. The differential effects of two lots of Aroclor 1254 on the selected neurochemical end points could not be clearly explained by the differences in their TEQ values. However, Burgin et al. (*39*) reported that TEQ predicted the effects on ethoxyresorufin-*O*-deethylase (EROD) or methoxyresorufin-*O*-deethylase (MROD) activities (strict dioxin-like effects), but not pentoxyresorufin-*O*-deethylase (PROD) or circulating serum thyroxine levels. These results suggest that different end points may be observed with different PCB mixtures. Hence, a mixture that is of low concern for one end point may be of greater concern for another end point. In addition, these results suggest that overall toxicity of complex mixtures can not be entirely predicted based on the TEQ values and caution should be used when making risk assessment decisions about chemical mixtures that involve both aryl hydrocarbon receptor-dependent and -independent mechanisms.

In general, current data indicate that the effects of two lots of Aroclor 1254 on selected neurochemical end points could not be explained either by total mass or by TEQ. These effects could not be attributed to the differences in the percentage of non-*ortho* congeners or dibenzofurans because they were inactive on these two parameters. These effects also could not be attributed to the interactions among the congeners and contaminants because our previous studies indicated that inactive congeners did not interfere with the activity of active congeners, and the interactions between two active congeners seem to follow additivity (*52*). However, these differential effects could be caused by differences in the composition of *ortho*-congeners in these two mixtures, because PCBs with *ortho*-lateral substitutions have been shown to exhibit different activities on the selected neurochemical end points (*43*). Because of these differential effects between different lots, the composition of Aroclor mixtures used in investigations should be disclosed.

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